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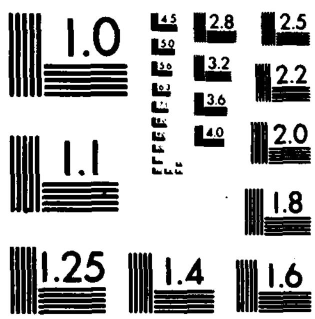

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Population dynamics and control studies on *Culicoides* sand flies were conducted at Parris Island, South Carolina, and Yankeetown, Florida.

Adult seasonal patterns were monitored by light traps. Four species, *C. furens* Poey, *C. hollensis* Melander and Brues, *C. melleus* (Coquillett), and *C. mississippiensis* Hoffman are considered abundant. *C. furens* and *C. melleus* are present from mid-April through late October; *C. hollensis* and *C. mississippiensis* peak in the spring and fall of the year. Larval habitat characterization

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20. ABSTRACT (Continued)

Studies were initiated. Fluctuations in larval density were correlated with plant cover.

Candidate insecticides were evaluated as larvicides, residual applications on household screens, and as aerosol adulticides. Based solely on toxicity to *Culicoides* larvae, the decreasing order of effectiveness was chlorpyrifos, temephos, fenthion, malathion, naled, and propoxur. Based on knockdown capability, toxicity, and longevity of the insecticides as a residual application on household screens, the decreasing order of effectiveness was propoxur, chlorpyrifos, malathion, and fenthion. Seven insecticides were evaluated in our wind tunnel screening as aerosol adulticides. In order of decreasing toxicity they were Decamethrin®, permethrin, resmethrin, *d*-phenothrin, naled, malathion, and fenthion. (trade name)

Four commercial products (Avon's Skin-So-Soft, Johnson's Baby Oil, Claubo, and mineral oil) were protective against sand fly bites. The mode of action was observed as trapping on oily skin rather than repelling attacking midges.

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(6) Evaluation of insecticides, insect growth regulators, skin and clothing repellents, and other approaches to the control of coastal sand flies, *Culicoides* spp.

by

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SUMMARY

Population dynamics and control studies on *Culicoides* sand flies were conducted at Parris Island, South Carolina, and Yankeetown, Florida.

Adult seasonal patterns were monitored by modified New Jersey light traps. Three species (*C. furens* Poey, *C. hollensis* Melander and Brues, and *C. melleus* (Coquillett) at Parris Island and 2 species (*C. furens* and *C. mississippiensis* Hoffman) at Yankeetown are considered major pests. *C. furens* and *C. melleus* have several peaks from mid-April through late October. Both *C. hollensis* and *C. mississippiensis* peak in the spring and fall of the year.

A detailed habitat characterization study has been initiated at Yankeetown. The relationship between larval population dynamics and factors such as time of year, tidal dynamics, vegetative cover, and soil factors is being studied. Larval counts made from extraction of larvae from weekly samples taken between May 28 and October 6 showed significantly greater numbers for *Distichlis* than for *Juncus* and *Spartina* vegetated areas.

Chemical studies with candidate insecticides include screening as larvicides, evaluating as residual applications on household screens, and laboratory screening as aerosol adulticides. Based solely on toxicity to *Culicoides* larvae, the decreasing order of effectiveness of 6 insecticides was chlorpyrifos, temephos, fenthion, malathion, naled, and propoxur.

Based on knockdown capability, high toxicity, and longevity of the insecticide as residual application on household screens, propoxur was the most effective chemical evaluated. Next in effectiveness was chlorpyrifos, followed closely by malathion. Fenthion was the least effective for all 3 factors, and demonstrated no practical use on window screens at any concentration tested.

Results of droplet size tests with malathion against field-collected *C. mississippiensis* indicate that aerosols from standard ground ULV generators with a volume median diameter (VMD) of 8-10 μm should be highly efficient for biting midge control. Seven insecticides were evaluated in our laboratory screening program as aerosol adulticides. Ranked in order of decreasing toxicity they were Decamethrin®, permethrin, resmethrin, *d*-phenothrin, naled, malathion, and fenthion.

Four commercial products (Avon's Skin-So-Soft, Johnson's Baby Oil, Claubo, and mineral oil) were field-tested as skin applications against 6 species of biting midges. All were effective as protectants against bites, however, the mode of action was observed to be trapping the midges on the oily skin surface rather than repelling attacking midges. In the absence of a suitable repellent any of these commercial materials might serve as useful substitutes to mechanically prevent bites.

I. INTRODUCTION

Because of the location of many military installations, particularly Navy bases, on coastal tidal areas in the continental United States and the Caribbean, and the widespread distribution of *Culicoides* sand flies (biting midges) in these areas, the midges pose a serious threat to the efficiency of personnel stationed at these bases. Historically, control of biting midges has been hampered by a lack of knowledge of their population dynamics and a lack of chemical products labelled for control of either immatures or adults. Nevertheless, there has been no sustained research effort to derive the data necessary for recommendations on insecticidal control and personal protection. This report contains the results of our first year's studies aimed at filling this information void. Studies were conducted on basic population dynamics, chemical control, and personal protection.

II. POPULATION DYNAMICS

Population studies of *Culicoides* were conducted at two sites: Parris Island Marine Recruit Depot, South Carolina, on the Atlantic coast, and Yankeetown, Florida, on the Gulf coast.

The Parris Island site is a good example of a type of salt marsh known as low, or regularly flooded marshes. The characteristic feature of this marsh is vast expanses of smooth cordgrass, *Spartina alterniflora* Lois., which grows in several height forms between mean sea level and mean high tide. Other characteristics of this site are high tidal amplitudes (>2.5 m), vast dendritic creeks, many deep tidal channels, and a very soft and silty marsh substratum.

At Yankeetown are found salt marshes typical of the entire Florida Gulf coast north of Tampa. The characteristic feature is broad expanses of black needle rush, *Juncus roemerianus* Scheele grading into upland. At this site *S. alterniflora* seldomly occurs in extensive stands, but is generally found fringing the edges of tidal creeks. Large patches of *Distichlis spicata* (Linnaeus Greene) are occasionally found at this site. Normally *J. roemerianus* grows just above the normal high tide line, but the Yankeetown marshes, like other northern Gulf coast marshes of Florida, have an unusual flooding pattern. From May through October the monthly mean sea level rises to more than a foot above the annual mean sea level; during this time even the *Juncus* and *Distichlis* areas are flooded by each high tide. During the rest of the year *Juncus* and *Distichlis* remain unflooded by even most spring tides.

At both study sites, adult *Culicoides* seasonal population patterns were monitored with New Jersey light traps, modified by replacing the standard delivery cone screen with 40-mesh brass screening. Data were obtained on species composition, seasonal incidence, and relative abundance. The light trap data (Tables 1 and 2) indicate that 3 species (*C. furens* Poey, *C. hollensis* Melander and Brues, and *C. melleus* (Coquillett) at Parris Island, and 2 species (*C. furens* and *C. mississippiensis* Hoffman) at Yankeetown are abundant enough to be considered as major pests. At both sites *C. furens* was present in trap collections from mid-April through late October with definite peaks in June, August, and late September - early October. *C. melleus* has a similar seasonal pattern. Both *C. hollensis* and *C. mississippiensis* are present in the spring and fall of the year with definite peaks in October through November, and March through mid-April.

At Yankeetown, larval population and habitat characterization studies were initiated near the end of May. A study area, located within a typical section of the salt marshes, is subdivided into eighty-five 25-m^2 plots. Each plot was characterized according to major vegetative cover type, *Spartina, Juncus, Distichlis,* and various mixtures of these grasses. Previous studies have shown that *C. furens* and *C. mississippiensis* utilize these intertidal plant communities as breeding locations. However, little is known about the relationship between the larval population dynamics of each of these *Culicoides* species, and other factors such as time of year, tidal dynamics, and various soil characteristics. Such information is very essential before control measures can be directed against immature stages. If larval distribution can be more definitively delimited, spatially and temporally, then larvicide treatments can be restricted to productive areas.

Sod samples (ca. 10 cm diam. X 8 cm deep) are taken weekly from each major vegetative cover type by means of post hole diggers. The location of each sample is determined through random selection of the plots in which the desired vegetation type is found. Larvae are extracted by our recently developed 2% agar technique (Kline et al. 1980). A portion of larvae recovered from each vegetative type is reared for sex and species identification.

The data (Table 3) collected between May 28 and October 6 show the mean number of larvae recovered from sod samples was significantly greater for *Distichlis* areas (7.46 per sample) than for *Juncus* and *Spartina* areas (5.64 and 4.75 per sample, respectively). Preliminary statistical analyses show that the factors DAY (week samples were taken), LOCATION (vegetative cover), and their interaction significantly explain ca. 30% of the variation in larval counts. We feel that a large portion of the remaining 70% variation may be explained by tidal dynamics and soil characteristics.

The identification of larvae recovered from soil samples and held for emergence shows a trend of decreasing *C. furens* and increasing *C. mississippiensis* (Table 4) during this sampling period. This trend substantiates light trap data (Table 2) which showed *C. furens* was more prominent in late spring through the summer months, and *C. mississippiensis* during late fall and winter months.

At Parris Island substrate sampling consisted of taking random samples from *Spartina* vegetation at different locations throughout the base. The number of samples taken during any week depended on the limited time available for sampling by our biologist stationed at this site. The data (Table 5) varied too much from samp' to sample, and from week to week to make many generalizations. It was noticed, however, that samples taken from underneath large oak tree trunks which had fallen into the marsh consistently produced large numbers of larvae with little variation. The 8 samples taken during the last sampling week (July 24-30) is a good example of this. Much more substrate sampling needs to be done at this site.

None of the larvae recovered were reared to adults, but 4 emergence traps were placed in the *Spartina* marsh and rotated weekly during the sampling period. Emergence trap data (Table 6) showed the same seasonal trends as the light traps (Table 1) with regard to *C. furens* and *C. hollensis*. No *C. melleus* were found in any of these collections. This is not surprising since past studies have shown that *C. melleus* prefers intertidal sandy beaches (Linley and Adams 1972; Kline and Axtell 1975). Much work needs to be done at Parris Island to more definitively delineate *Culicoides* larvae distribution and characterize the breeding habitats of the major speices.

III. CHEMICAL CONTROL STUDIES

Chemical studies have been divided into 3 main areas with candidate insecticides: screening as larvicides, evaluation of residual applications on household screens, and laboratory screening as aerosol adulticides.

Larvicide Screening

Larvae for these tests were obtained from field-collected substrate samples taken from known breeding areas at Yankeetown. Larvae were extracted from the substrate by the agar extraction method previously cited. Only 3rd and 4th instar larvae were used in these tests. The larvae were transferred with an angled stainless steel probe into a 50 ml glass beaker containing 25 ml of untreated estuarine water. This beaker was then decanted with a swirling action into a 250 ml glass beaker containing 100 ml of estuarine water that is either untreated, or treated at predetermined discriminating concentrations (ppm) of insecticide. Since the insecticides are formulated in acetone, the checks are also conducted with acetone-water solutions. For each test, duplicate beakers are treated at each concentration level, thus exposing 20 larvae at each level. Each test is replicated at least 5 times.

At 24 hr postexposure mortality counts are made. Larvae are considered dead if, when touched with a probe, they lack the ability to move in their characteristic serpentine motion, i.e., rapid flexing of the body.

Since the larvae are found in sod, the effects of habitat sod placed in the treatment water also were studied. The dose-response relationship is determined by probit analysis of the log-transformed mortality data.

During this past year, 6 insecticides were evaluated as larvicides: temephos, propoxur, fenthion, naled, malathion, and chlorpyrifos. Based solely on their toxicity (Table 7) to biting midge larvae, chlorpyrifos and temephos were an order of magnitude more effective than the other chemicals, followed in order by fenthion, malathion, naled, and very distantly by propoxur. Addition of habitat soil did not change the order of effectiveness, but 3-4 times as much temephos and chlorpyrifos and ca. 1.25 - 1.5 times as much fenthion, malathion, and naled were required to cause the same mortality as estuarine water alone. Habitat soil had little or no effect on the killing effect of propoxur.

Evaluation of Treated Window Screens

The evaluations of residual applications of candidate insecticides were conducted against *C. mississippinesis* using 2 different methods: a small screen method used previously by several investigators (Jamnback 1961, 1963; Dukes and Axtell 1976), and a newly developed large screen method (Roberts and Kline 1980). Aluminum screening, 16 X 18-mesh, was used in both methods.

For both methods, insecticides were tested in concentrations of 1, 3, 5, and 8% active ingredient (weight/volume). The insecticide solutions were agitated thoroughly before the treatment period. Screens used in these tests were treated by dipping in an acetone solution of technical insecticide or, in the case of emulsifiable concentrates, in a water solution for about 10 seconds After treatment, the screens were hung beneath the eaves of a building to dry. Control tests were conducted with screens immersed in acetone only.

During the past year chlorpyrifos, fenthion, malathion, and propoxur, all formulated in acetone with technical grade material, were tested by the small screen method. For large screen tests chlorpyrifos and propoxur were tested as emulsifiable concentrates, and as technical grade material in acetone.

The small screen method uses a divided test chamber that consists of two 500 ml paper cartons with the open tops abutting and taped together with a 9 cm diam. screen inserted in the middle. The bottom of one of the containers was replaced with transparent plastic to allow light to enter and the bottom of the opposite container was left intact to create partial darkness during testing. A hole ca. 2.5 cm in diameter is made in the bottom of the other carton and closed with a stopper. Total darkness was created by covering the plastic window with a lid.

Adult biting midges used in these small screen tests were collected from natural populations by either inducing them with CO_2 to enter a field trailer and subsequently aspirating them off a window, or attracting them into a CO_2-baited suction box trap immediately before testing. While in the box trap the biting midges had access to 10% sugar water. Approximately 30 midges were introduced into the chamber through the stoppered end. All tests consisted of at least 4 replicates, but as many as 8 replicates were obtained for some chemical concentrations.

When the lid was removed the biting midges, attracted by light, quickly passed through the treated screen. Lighting was standardized by placing a 25-watt fluorescent fixture ca. 8 cm in front of the test chambers. After passing through the treated screens, the midges were lightly anesthetized with CO_2 and transferred to holding cages which consist of a 215 cc. paper carton with a fine mesh cloth as a top. Cotton pads soaked in 10% sugar water solution were placed on top of the holding cages to provide food and water for the midges during the holding period. The holding cages were placed in large styrofoam chests, containing moistened cotton pads to maintain a high humidity environment, and moribund and dead adults were counted at 0.5, 1, 2, 3, 4, and 24 hr postexposure.

After each test, the treated screens were removed from the test chamber and hung outside under the eaves of a building to age and weather. The screens were tested ca. every 7 days, depending on the availability of adult biting midges, to determine the longevity of each treatment.

The large screen technique utilizes the biting midges attractancy to CO_2 and light to induce natural (field) populations of biting midges to pass through a treated screen into a collecting cage. Essentially, the trap is a box shell 30.5 cm (H X D X W) except for one side which was left open for insertion of a large 950 cm^2-treated screen. On the side directly opposite the treated screen, a small hole is cut for attachment of a collection cage. During testing the trap is oriented so that the screen is nearly horizontal ca. 20-30 cm above ground level, and the collection cage is almost perpendicular with the ground. CO_2 is released (ca. 100 ml/min) at ground level below the trap from a compressed gas tank with a flow rate controlled by means of a single stage regulator and metered with a Gilmont® compact flow meter. Four exposures of at least 25 biting midges each are made for each treated screen. As with the small screen, counts of moribund or dead midges are made at 0.5, 1, 2, 3, 4, and 24 hr. after exposure. After completion of the tests the boxes are left in the collection area for aging and weathering with screens in place but in a vertical position. Effectiveness of the candidate insecticide is tested at various intervals after treatment, depending on the availability of natural populations of adult biting midges.

Criteria for effectiveness were knockdown capability (1 hr mortality count), high toxicity (4 hr mortality count), and longevity of the insecticide when weathered.

Based on these 3 criteria, propoxur (Tables 8-10) was the most effective chemical tested. At 8% propoxur caused 87 - 100% knockdown, and 95-100%

high toxicity for 35 days after treatment. After propoxur, the most promising chemical was chlorpyrifos, followed closely by malathion. At 8% both were nearly equally effective, with malathion having a slight edge in knockdown capability, and chlorpyrifos with an edge in longevity of high toxicity. Fenthion was the least effective for all three factors, and demonstrated no practical use on window screens at any concentration tested.

In Table 9 the results of testing emulsifiable concentrate formulations in water at 8% are presented for chlorpyrifos, propoxur, and malathion, as well as the 8% technical in acetone formulation for malathion. Based on these data we have concluded that water-based formulations of emulsifiable concentrates are not as effective as residual window screen treatments as technical in acetone formulations.

Future evaluations of candidate insecticides for residual screen treatments will be a 2-step testing program in which we plan to use the small screens for initial testing of new materials and the large screens for secondary evaluation under more natural conditions. Secondary evaluations will also include different formulations and methods of application.

Aerosol Adulticide Studies

Droplet-size studies.--In mosquito control, droplet size of an insecticide aerosol influences efficiency in killing adult mosquitoes (Mount et al. 1975). To determine the effect of droplet size on insecticidal efficiency on biting midges, laboratory wind tunnel tests were conducted with caged adult female *C. mississippiensis*. A Berglund-Liu Monodisperse Aerosol Generator (Thermo Systems, Inc.,) was used to introduce uniform sized droplets into a wind tunnel for exposure of insect samples.

Droplet size was varied by a technique involving evaporation of a volatile solvent (isopropanol) from a solution containing a nonvolatile insecticide (malathion) at different concentrations. Exposure time was varied to provide different dose levels.

A series of tests with field-collected *C. mississippiensis* adult females were conducted for 3 aerosol sizes (2.8, 4.8, and 8.2 μm diameters) of malathion at 6 dose levels. Mortality readings were made at 1, 2, and 24 hr. after treatment.

Each treatment was replicated 4 times on different days. The results (Table 11) showed an increase in mortality (lower LD-50) with time after treatment due to a slow response to malathion. The smallest aerosol size (2.8 μm) was clearly less efficient than the 2 larger sizes (95% fiducial limits do not overlap at 2 and 24 hr posttreatment). The largest size (8.2 μm) produced the lowest LD-50 at each time, however, the difference between 4.8 and 8.2 μm was not large (95% fiducial limits overlap at each reading). The leveling-off tendency between 4.8 and 8.2 μm indicates that the optimum size is not much, if any, greater than 8.2 μm. Further tests with larger sizes will be necessary to confirm this. The results of these tests indicate that aerosols from standard ground ULV generators with a volume median diameter (VMD) of 8-10 μm should be highly efficient for biting midge control.

Relative toxicity tests.--We feel that compounds registered for ground ULV application for mosquitoes, if used properly, can give some temporary local reductions in biting midges. However, insufficient studies have been conducted to provide adequate data on control by this method. Therefore, the evaluation of candidate chemicals against adult *Culicoides* in wind-tunnel tests is an essential part of our research program.

The wind tunnel system used was basically that described by Mount et al. (1976). Several changes were necessitated by the small size of the adult biting midges. The major change was the substitution of the 16-mesh galvanized screen wire wind tunnel tube with 40-mesh brass screen. Also the fabricated atomizing nozzle was changed to a commercially-available standard stock nozzle (#12891 1/8JJ, Spraying Systems Co., Chicago, IL). The wind tunnel consists of a cylindrical tube 15.5 cm in diameter through which a column of air is blown at a rate of 6.4 km/ha. Approximately 25 adult female biting midges are confined in cardboard exposure cages, 8.6 cm in diameter and 5.0 cm high, with 40-mesh brass screen ends. The cages are placed in the center of the tube for exposure. One-fourth ml solution of the desired concentration of the technical insecticide in acetone (wt A.I./volume diluent and expressed as % concentration) is atomized at 105.5 g/cm^2 into the upwind portion of the tube, and the insects are exposed momentarily as the aerosol passes through the cage. We use an automatic pipette for convenience and efficiency.

Following exposure, the insects are lightly anesthetized with carbon dioxide and transferred to new 8.6 X 5.0 cm cardboard holding cages covered with fine mesh cloth screen tops. Cotton pads, soaked in a 10% sucrose solution, are placed on the cloth screens to sustain the insects until all mortality counts are made. The holding cages are placed in large styrofoam chests, containing moistened cotton pads to maintain a high humidity environment. Knockdown is checked 1 hr after treatment, and mortality is recorded 24 hr after treatment. Checks are exposed to contact sprays containing acetone only and handled in the same manner.

In our testing procedure candidate insecticides are first tested at concentrations of 0.25% (wt/v). Then the concentration is successively reduced by one-half until the 24-hr mortality falls below 50%. Pipettings of a serial dilution of a given insecticide are made from low to high concentrations without cleaning the wind tunnel. However, the tunnel is cleaned at the beginning and end of each test series and before each new insecticide is tested.

Since we do not have a laboratory colony of *Culicoides*, field collections of adults are made with CO_2-baited suction traps. The biting midges are transported to the laboratory and knocked down; ca. 25 are transferred to each exposure cage within a cold room. Duplicate cages are used in each test and at least 3 replicates are made with each concentration of each insecticide. At least 4 discriminating concentrations of each insecticide are used in each replicate.

This test is not intended to yield results that can be compared closely; however, it does eliminate those insecticides that are not toxic enough to warrant further testing, and it identifies the range of discriminating concentrations for those insecticides that are highly effective. This information is useful in the design of additional test programs.

The dosage/mortality data are used to determine the LC-50 and LC-90 of each compound. The dose-response relationship is determined by probit analysis of the log-transformed mortality data.

During this past year, 4 synthetic pyrethroid (Decamethrin®, permethrin, resmethrin, and *d*-phenothrin), and 3 organophosphorous (naled, malathion, and fenthion) compounds were evaluated in our laboratory screening program.

The 24 hr LC-50's and LC-90's and their respective 95% fiducial limits are presented in Table 12. The compounds are ranked in order of decreasing toxicity (LC-90) to *C. mississippiensis*. The synthetic pyrethroids were by far the most effective insecticides. Of the 3 organo-phosphorous compounds tested, naled was slightly more effective than malathion. Fenthion was the least effective of all the insecticides tested.

Relatively quick knockdown is a desirable characteristic of biting midge adulticides. All the pyrethroids produced relatively quick knockdown (within 1 hr) at concentrations near that required for 24-hr mortality. Naled produced the best knockdown among the 3 O-P compounds. Fenthion demonstrated practically no knockdown within 1 hr, and malathion showed only moderate knockdown.

IV. PERSONAL PROTECTION

Prior to initiation of the present contract, we (Schreck et al. 1979) had field tested the effectiveness of deet-treated net jackets against 4 species of biting midges (*C. furens*, *C. mississippiensis*, *C. hollensis*, and *C. barbosai* Wirth and Blanton). We found that the jackets provided ca. 98% protection against all species except *C. barbosai*. They gave only ca. 59% protection against this species.

During this past year 4 commercial products were field-tested as skin applications against 6 species of biting midges. Tests were designed to evaluate Avon's Skin-So-Soft, Johnson's Baby Oil, Claubo, and mineral oil using deet as the standard.

The test procedures were described by Schreck et al. (1979). The commercial formulations and mineral oil were applied at full strength whereas a deet standard was applied at a 12.5% or 25% ethanol solution. All applications were made in 1 ml aliquots and spread evenly over the forearm of a subject from wrist to elbow. Six subjects participated in each of the studies.

All subjects wore a headnet and shirt with sleeves rolled to the elbow. Gloves were worn by the treated subjects, permitting exposure of only the forearm whereas exposure of the hands of the check subject allowed extra freedom for killing and counting biting midges. All wore long pants, usually military fatigues. Treated arms were continuously exposed to the natural population of midges. Tests were made in units of 5 min each because of the fluctuating activity of the midges. Up to 21 5-min tests were made in 1 replication of a test series, depending on midge activity and weather conditions at each location.

The purpose of the 5-min test was to establish an index of biting pressure by using the untreated check subject as an indicator of activity while the other subjects concurrently exposed treated arms to the midge population. At the end of 5 min, the number of bites occurring on each of the test subjects and the check were recorded. The subjects (each separated by 8 - 10 m) then changed positions, moving in a clockwise direction so that no one remained at a location longer than 5 min. A different subject was used as a check in each replication of the study. During each 5-min test, the check subject counted only those midges that were biting the forearms and hands, and these were crushed so that they were not counted more than once. Each material was paired directly with deet in each test series. Since it was not always possible to distinguish species when they were biting, collections were made by aspirating midges in the act of biting the untreated check subject during tests. Subsequent determination of species composition of the biting midge population was then made.

Results of the study (Table 13) show Skin-So-Soft, Johnson Baby Oil, Claubo, and mineral oil are effective as protective applications for the prevention of bites from at least 6 species of the genus *Culicoides*. Certainly, in the absence of a suitable repellent these commercial materials might serve as useful substitutes to mechanically prevent bites. Observations made by all test subjects revealed that whereas the mode of action of deet was to repel attacking midges, the other materials merely trapped them on the oily skin surface, thus preventing bites.

As a caution, it must be noted that Skin-So-Soft is marketed not as a skin application at full strength but as a bath oil at the rate of 1/2 cap full in a bath tub of water. It would therefore be prudent to investigate what effect, if any, long-term skin applications might have on the potential user of this as well as the other materials tested.

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Table 1.--Avg. no. (per trap/night) of *Culicoides* spp. collected at 4 locations at Parris Island, South Carolina.

Location		Number of *Culicoides*											
		Horse Island			Elliott Beach			Rifle Range			Gardens		
Species a/		f	h	me	f	h	me	f	h	me	f	h	me
Date													
Mar.	13-19	0	13	0	0	0	0	0	0	0	0	16	0
	20-26	0	377	0	0	0	0	0	0	0	0	300	0
Mar/Apr	27-02	0	3,735	2	0	20,745	0	0	723	0	0	179	0
	03-09	0	1,911	0	0	48,143	2,143	0	7,121	32	0	157	6
	10-16	5	8,996	1,411	21	27,570	4,896	6	2,983	261	0	374	35
	17-23	28	1,950	239	315	16,258	3,452	90	4,530	762	7	201	16
	24-30	8	28	92	136	388	46	43	109	50	15	37	15
May	01-07	49	218	245	12,953	21,843	2,507	21,634	38,396	3,430	107	315	20
	08-14	276	649	200	336	304	27	41	51	7	9	12	2
	14-21	1,738	2,138	551	2,129	1,061	71	96	102	19	16	19	0
	22-28	332	5	59	1,821	34	24	380	127	254	15	2	0
May/Ju.	29-03	182	4	89	496	9	19	31	4	14	11	1	2
	05-11	32	1	20	194	12	34	114	11	24	7	0	1
	12-18	3	0	2	31	1	1	30	1	5	2	0	2
	19-25	7	0	2	193	1	40	17	0	3	6	0	1
Jun/Jul.	26-02	14	0	4	534	0	56	63	0	15	4	0	0
	03-09	12	0	3	1,732	0	18	14	0	2	4	0	1
	10-16	8	0	1	192	0	4	15	0	2	4	0	0
	17-23	8	0	1	259	0	2	6	0	2	2	0	0
	24-30	16	0	3	2,108	0	7	59	0	9	34	0	2
Jul/Aug	31-06	7	0	1	171	0	7	9	0	0	1	0	0
	07-13	1	0	1	966	0	1	6	0	0	8	0	0
	14-20	1	0	0	1,129	0	96	91	0	29	8	0	0
	21-27	1	0	0	258	0	4	26	0	9	21	0	0
Aug/Spt	28-03	3	0	2	290	0	4	23	0	0	2	0	0
	04-10	14	3	7	3,571	18	111	97	9	22	30	538	3
	11-17	8	1	3	1,036	10	227	29	5	3	14	2	0
	18-24	42	9	6	1,037	8	18	1,469	24	11	3,393	105	17
	25-30	6	1	0	149	9	1	130	24	3	63	17	10

a/ f = *furens*; h = *hollensis*; me = *melleus*.

Table 2.--Avg. no. (per trap/night) of *Culicoides* spp. collected at 3 locations at Yankeetown, Florida

Location / Species[a]	Number of *Culicoides*								
	Trailer			Bonita Club			Cypress Marina		
	mi	f	b	mi	f	b	mi	f	b
Dates									
1979									
Sept/Oct 26-03	9	164	0	111	209	70	13	46	0
Oct 04-10	42	99	6	248	296	181	40	54	1
11-17	35	35	17	114	53	86	28	24	1
18-24	105	18	1	1,439	250	55	331	48	1
25-31	7	0	1	9	0	1	68	2	0
Nov 01-07	193	1	2	68	1	8	86	1	0
08-14	65	0	0	92	1	6	1	0	0
15-21	3	0	0	0	0	0	0	0	0
22-28	38	0	0	90	0	0	3	0	0
Nov/Dec 29-05	5	0	0	0	0	0	0	0	0
Dec 05-12	75	0	0	25	0	0	76	0	0
12-19	29	0	0	53	0	0	149	0	0
20-26	34	0	0	10	0	0	57	0	0
1980									
Dec/Jan 27-02	0	0	0	1	0	0	41	0	0
03-09	4	0	0	1	0	0	43	0	0
10-16	112	0	0	4	0	0	139	0	0
17-23	45	0	0	1	0	0	81	0	0
24-30	109	0	0	7	0	0	128	0	0
Jan/Feb 31-06	7	0	0	4	0	0	3	0	0
Feb 07-13	1	0	0	0	0	0	6	0	0
14-20	730	0	0	17	0	0	49	0	0
21-27	327	0	0	189	0	0	202	0	0
Feb/Mar 28-05	126	0	0	18	0	0	109	0	0
Mar 06-12	364	0	0	4	0	0	156	0	0
13-19	1,499	0	0	240	0	0	1,146	2	0
20-26	1,957	0	0	143	[illegible]	0	514	0	0
Mar/Apr 27-02	686	0	0	1,356	1	0	857	4	0
Apr 03-09	1,127	9	5	149	2	0[b]	49	1	0
10-16	150	1	0	--	--	--[b]	98	1	0
17-23	208	9	3	--	--	--	10	0	0
24-30	30	3	3	1,009	13	15	132	0	0

Table 2.--Continued

Location / Species a/	Trailer mi	Trailer f	Trailer b	Bonita Club mi	Bonita Club f	Bonita Club b	Cypress Marina mi	Cypress Marina f	Cypress Marina b
Dates									
1980 (Cont.)									
May 01-07	15	11	0	843	67	39	--	--	-- b/
08-14	8	10	0	300	313	56	--	--	-- b/
15-21 c/	42	5	0	1,392	256	712			
22-28	0	0	0	10	7	6			
May/June 29-04	1	1	0	2,253	535	50			
June 05-11	--	--	-- b/	88	164	38			
12-18	--	--	-- b/	305	425	9			
19-25	0	0	0	0	0	0			
Jun/July 26-02	0	2	0	0	0	0			
July 03-09	0	0	0	0	1	0			
10-16	0	3	0	--	--	-- b/			
17-23	0	1	0	1	132	4			
24-30	0	0	0	--	--	-- b/			
July/Aug (2 wk) 30-13	0	387	0	0	674	0			
Aug 14-20	0	169	10	0	332	0			
21-27	1	595	5	0	78	1			
Aug/Sept 28-03	0	126	3	3	1,009	22			
Sept 04-10	0	96	0	--	--	-- b/			
11-17	--	--	-- b/	--	--	-- b/			
18-24	0	10	0	28	1,488	36			
Sept/Oct 25-01	0	10	1	--	--	-- b/			
Oct 02-08	0	8	1	0	0	0			
09-15	9	34	0	73	50	5			
15-22	7	4	1	35	9	16			

Column heading above all locations: Number of *Culicoides*

a/ mi = *mississippiensis*; f = *furens*; b = *barbosai*.

b/ Trap malfunctioned.

c/ Cypress marina light trap removed because of repeated vandalism.

Table 3.--Mean no. *Culicoides* **larvae recovered from soil samples taken in major vegetative zones, Yankeetown, Florida, 1980.**

Location	Number of larvae recovered																			
	28 May: 149	4 Jun: 156	11 Jun: 163	18 Jun: 170	25 Jun: 177	30 Jun: 182	7 Jul: 189	14 Jul: 196	21 Jul: 203	28 Jul: 210	6 Aug: 219	18 Aug: 231	25 Aug: 238	2 Sept: 246	8 Sept: 252	15 Sept: 259	22 Sept: 266	6 Oct: 280	$\bar{X}$	a/
Distichlis	22.4	1.7	9.5	0.9	4.7	6.8	7.2	4.2	18.5	7.4	4.5	4.2	4.2	11.5	5.6	5.1	7.7	8.2	7.46	a
Juncus	1.0	1.0	5.8	5.6	2.5	3.4	12.3	10.6	13.5	6.2	3.1	9.0	5.3	2.7	3.9	7.6		2.3	5.64	b
Spartina	.5	.8	2.8	21.7	2.3	1.6	2.9	3.8	9.8	2.6	2.0	3.1	5.9	2.0	3.9	7.6	5.8	6.4	4.75	b

a/ The means with a common letter are not significantly different at the 0.5 level (Duncan's Multiple Range Test).

Table 4.--Percentage of *Culicoides furens* and *Culicoides mississippiensis* emerged from larvae recovered from soil samples.

Species	Distribution of species in soil samples (%)																
	28 May 149	4 Jun 156	11 Jun 163	18 Jun 170	25 Jun 177	30 Jun 182	7 Jul 189	14 Jul 196	21 Jul 203	28 Jul 210	6 Aug 219	18 Aug 231	25 Aug 238	2 Sept 246	8 Sept 252	15 Sept 259	22 Sept 266
C. furens	--	--	100	100	85.7	70	--	70	76	71	--	44	42	66	50	50	12
C. mississippiensis	--	--	0	0	14.3	30	--	30	24	29	--	56	58	34	50	50	88

Table 5.--Recovery of *Culicoides* larvae from substrate samples taken from *Spartina* habitats at Parris Island, South Carolina.

Dates		Number samples	Number pos. samples	Range of pos. samples	Avg. no. larvae per sample
March	19-25	6	6	1-200	65.3
March/April	26-02	4	4	1- 9	0.8
April	03-09	26	4	1- 7	0.7
	10-16	--	--	--	--
	17-23	15	9	1- 18	2.7
	24-30	12	8	1- 18	4.7
May	01-07	7	4	1- 2	0.9
	08-14	9	6	1- 30	6.2
	15-21	4	3	6- 19	8.0
	22-28	8	6	2- 21	9.1
May/June	29-04	4	3	2- 5	2.3
June	05-11	12	8	1- 7	2.0
	12-18	--	--	--	--
	19-25	--	--	--	--
June/July	26-02	4	3	1- 4	1.8
July	03-09	8	4	1- 12	2.1
	10-16	4	3	1- 7	3.5
	17-23	4	3	5- 12	7.3
	24-30	8	8	10- 75	43.4

a/ All samples taken from under fallen tree trunks.

Table 6.--Seasonal emergence pattern of *Culicoides* spp. from *Spartina* habitats at Parris Island, South Carolina.

Dates	Number collected	
	C. hollensis	*C. furens*
March/April 26-02	15	0
April 03-09	13	0
10-16	8	0
17-23	36	0
24-30	25	0
May 01-07	16	5
08-14	41	10
15-21	33	7
22-28	23	54
May/June 29-04	--	--
June 05-11	4	15
12-18	3	2
19-25	4	59
June/July 26-02	0	47
July 03-09	0	23
10-16	0	93
17-23	0	2
24-30[a/]	0	85

Table 7.--Toxicity of candidate chemicals as larvicides against *Culicoides mississippiensis* Hoffman.

Chemical	Water only		Water/habitat soil	
	LC-50	LC-90	LC-50	LC-90
Chlorpyrifos	.001	.002	.003	.007
Temephos	.002	.006	.013	.025
Fenthion	.011	.023	.014	.032
Malathion	.121	.402	.198	.601
Naled	.418	.826	.500	1.036
Propoxur	3.348	7.944	4.000	7.924

Table 8.--Knockdown effectiveness of small treated screens (technical in acetone) against *Culicoides mississippiensis* Hoffman at Yankeetown, Florida.

Chemical	Conc. (%)	Avg. % mortality[a,b] (1 hr postexposure) at days treated screens were weathered: 1	7	14	21	28	35
Propoxur	1	92	66	69	16	4	3
	3	93	98	82	41	10	2
	5	100	100	93	76	64	64
	8	100	98	100	96	87	90
Chlor-pyrifos	1	50	NT[c]	1	10	2	--
	3	74	NT	33	32	7	--
	5	88	NT	82	63	50	8
	8	93	NT	90	74	64	48
Malathion	1	41	15	20	11	2	6
	3	87	45	47	27	3	3
	5	87	55	53	39	40	18
	8	98	93	97	76	58	53
Fenthion	1	5	4	3	2	2	1
	3	11	1	3	1	1	1
	5	27	2	6	5	2	2
	8	47	11	12	5	8	6
Checks		2	2	3	2	2	2

a/ Mortality data are corrected for control by Abbott's formula.

b/ Avg. of 4-6 replicates of ca. 25 flies each.

c/ Insufficient natural populations for testing.

Table 9.--Toxic effectiveness of small treated screens (technical in acetone) against *Culicoides mississippiensis* Hoffman at Yankeetown, Florida.

Chemical	Conc. (%)	Avg. % mortality[a,b] (4 hr postexposure) at days treated screens were weathered: 1	7	14	21	28	35
Propoxur	1	95	69	69	17	2	5
	3	95	98	82	50	11	1
	5	100	100	98	81	67	75
	8	100	100	100	100	95	97
Chlor-pyrifos	1	83	NT[c]	47	47	27	--
	3	89	NT	80	62	36	--
	5	99	NT	98	93	73	73
	8	99	NT	100	99	97	97
Malathion	1	64	22	27	15	4	6
	3	94	58	58	27	3	15
	5	99	70	69	39	43	34
	8	99	99	98	87	79	80
Fenthion	1	35	17	3	1	3	1
	3	63	22	12	6	1	4
	5	77	23	24	21	2	3
	8	83	83	77	33	52	31
Checks		3	4	5	4	10	7

a/ Mortality data are corrected for control by Abbott's formula.

b/ Avg. of 4-6 replicates of ca. 25 flies each.

c/ Insufficient natural populations for testing.

Table 10.--Effectiveness of large treated (8% wt/vol) screens against *Culicoides mississippiensis* Hoffman at Yankeetown, Florida.

Chemical	Formulation[b/]	Avg. % mortality (4 hr. postexposure) at days treated screens were weathered[a/]				
		1	7	14	21	35
Malathion	t	95	92	88(est.)	--	77
	EC	75	51	62	--	76
Propoxur	EC	74	52	29	--	--
Chlorpyrifos	EC	99	89	72	66	49

a/ Avg. of 4-6 replicates of 25 flies each.

b/ t = Technical insecticide in acetone.
EC = Emulsifiable concentrate; water-based formulation.

Table 11.--Effect of droplet size on dose units of malathion required for 50% mortality of caged *Culicoides mississippiensis* Hoffman exposed to wind tunnel generated aerosols.

Aerosol droplet (μm)	LD-50[a] (95% fiducial limits)		
	1 hr. posttreatment	2 hr. posttreatment	3 hr. posttreatment
2.8	13.6 (9.63 - 19.56)	8.60 (7.55 - 9.84)	3.53 (3.03 - 4.08)
4.8	9.80 (8.48 - 11.43)	4.93 (4.29 - 5.65)	1.86 (1.07 - 2.69)
8.2	8.17 (5.83 - 11.94)	4.69 (4.01 - 5.46)	0.96 (0.68 - 1.24)

a/ LD-50 is presented in arbitrary dose units (1 unit = 0.0066 μg technical malathion).

Table 12.--Effectiveness of seven insecticides in laboratory wind tunnel tests against field-collected adult female *Culicoides mississippiensis* Hoffman.

Insecticide	24 hr. LC-50[a]	95% Fiducial limits		24 hr. LC-90	95% Fiducial limits	
		Lower	Upper		Lower	Upper
Decamethrin®	.00005	.00004	.00006	.00087	.0063	.00136
Permethrin	.00034	.00020	.00049	.00487	.00310	.00983
Resmethrin	.00115	.00071	.00159	.01134	.00832	.01781
d-Phenothrin	.00224	.00016	.00125	.03027	.01156	.03688
Naled	.01341	.00942	.02958	.17710	.03163	.21458
Malathion	.02395	.01469	.03336	.21206	.13198	.49897
Fenthion	.02200	.01973	.02449	.53387	.41226	.72148

a/ Expressed as % concentration - (wt A.I/volume diluent).

Table 13.--Mean numbers of bites/min received from *Culicoides* spp. in paired tests with a deet standard as a 12.5 or 25% ethanol solution and the candidate materials at full strength.

Paring of candidates and standard	Yankeetown FL[a/]		Ft. Myers FL[b/]		Parris Island SC[c/]	
	No. of repli- cations	Mean no. of bites	No. of repli- cations	Mean no. of bites	No. of repli- cations	Mean no. of bites
Deet	6	0	3	1.46	3	.007
Skin-So-Soft	6	0.06	3	0.25	3	0
Deet	4	0	2	.13	3	.007
Johnson's Baby Oil	4	.01	2	2.0	3	.003
Deet	4	0	2	.75	3	.093
Claubo	4	.38	2	.25	3	0
Deet			2	.25	3	.003
Mineral oil			2	.38	3	0
Check (total of 2 arms)		12.8		83.7		11.7

a/ Species identified in biting collections were 58% *C. mississippiensis*, 17% *C. barbosai*, 13% *C. floridensis*, and 12% *C. furens*.

b/ Species identified in biting collections were 94% *C. barbosai* 5% *C. furens*, and 1% *C. floridensis*.

c/ Species identified in biting collections were 80% *C. hollensis* and 20% *C. melleus*.

d/ Deet tested at 25% of ETOH.

e/ Deet tested at 12.5% in ETOH.